A Model for the Regeneration of Axons in a Nerve Guide Conduit (NGC) Used to Treat Peripheral Nerve Injuries

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Abstract

Nerve guide conduits are key devices in the treatment of peripheral nerve injuries that help direct the growth of damaged axons. Modern nerve guide conduits still use a simple design that lacks any growth factors, and experiments into improved designs have too many parameters to test. In order to narrow down the parameters of interest, this report implements a model of axonal growth cone regeneration comprised of a COMSOL model of growth factor diffusion and an SDE model of concentration and gradient dependent growth cone movement. From this model, it was found that increasing the growth factor concentration had a small impact on the straightness of, and therefore the speed of, axon regeneration while the overall concentration had a large impact on axon growth speed. From this, it can be concluded that experiments into improved nerve guide conduit designs may benefit more from a focus on providing a large concentration of factors than on introducing a large growth factor concentration gradient if the objective is a faster rate of recovery.

Background

Globally, more than one million people suffer from peripheral nerve injuries (PNIs) every year[1]. In the United States alone close to 560,000 procedures to treat PNIs are conducted on a yearly basis[2]. Current surgical treatment options include nerve end-to-end repair, autografts, allografts, and nerve guide conduits (NGCs)[3]. Of the aforementioned methods, NGCs have the most room for refinement and innovation to improve peripheral nerve treatment outcomes. NGCs are hollow cylindrical tubes used to bridge the nerve discontinuity present in PNIs. They are commonly made of Type 1 Collagen, poly-lactic acid, or poly-lactic-co-glycolic acid. NGCs have demonstrated efficacy in shorter nerve gaps (<20mm), but drops in the standard of care when treating larger nerve gaps[4].

It is accepted that nerve regeneration in current NGCs follows consecutive steps. First a fibrin bridge forms across the injury site to connect the proximal and distal nerve ends. Schwann cells (SC) proliferate to the fibrin cable and align longitudinally to form cell strands called bands of Büngner[5]. These bands can be better described as microchannels that are crucial pathways for selective axonal regeneration to occur. By providing a model to describe nerve regeneration in a nerve guide conduit following this widely accepted mechanism we can better describe the shortcomings of current NGCs and therefore how these can be improved with biomolecule supplementation. This model could describe where and when biomolecule supplementation would be most advantageous to increase rate and straightness of axons growing in an NGC. By providing a model that successfully describes this, it could become a stepping stone to explain the effect of intraluminal framework, the effect of its geometry, biomaterial composition, density etc. This would effectively reduce the experimental burden that accompanies NGC enhancement studies; and in the future could answer many questions, such as the effect of an intraluminal structure to support or replace the fibrin bridge as it is still unclear if they can be present in tandem.

Previous experiments have provided a significant amount of information for the understanding and modeling of axonal growth. *Razetti et al.* provide a model for single axonal path generation and collective axon growth[6]. The focus of the model is to take into account the complex mechanical interactions between axons as well as branching in a crowded environment. Axonal paths are determined in 3D using the successive addition of discrete segments with a fixed 1 μm length. Each step is said to follow a Markov chain in that its orientation depends on that of the previous step as well as the directionality of an external attractive field. The model relies on two main parameters α (axon rigidity or resistance to bending when growing) and β (axon attraction to an external attractive field). The parameters were determined from reconstructions of real axons from a *Drosophilia*'s (fruit fly) brain. The angles (in the xy plane and z plane) of axonal growth are determined stochastically at each step relying on a conditional Normal probability distribution that is a function of α and β (axon resistance to bending and attraction to an external attractive field respectively). Branching occurs

stochastically in this model if after a certain number of steps, a random number from 0 to 1 is smaller or equal to a branching probability parameter (that needs to be determined empirically). The combination of a deterministic component (Markov property) with a stochastic component in axonal growth is relevant to a model for peripheral nerve regeneration. The branching probability aspect of the model relies heavily on experimental values that would need to be drawn from human peripheral nerves for clinical accuracy of the model. We expect that axonal growth in a peripheral nerve need be modeled differently from a brain; especially when introducing the physical boundaries of a nerve guide conduit. Longitudinal growth along a nerve cable is different from a tree-like brain structure. Additionally, this model does not include a standard axonal growth rate, and relies on fixed length which should be refined.

Another model of interest was developed by *Borisyuk et al.* in describing the 2D longitudinal growth of axons in the spinal cord of tadpoles[7]. The model relies on three equations that describe growth in the x-direction, growth in the y-direction, and the growth angle. Growth is modeled in 1 μm steps where the change in x and y relies on an angle θ. This model integrates a deterministic component to the change in growth angle θ based on parameters μ and γ.$\mu$is analogous to the previous β, it describes the effect of an external attractive field on axonal growth direction. γ is a parameter that describes the propitious growth of axons in a set longitudinal direction. Although the paper does not describe what is meant by “longitudinal growth”, we believe this parameter to explain that an axon constrained to grow in a specific direction will tend to persistently grow in that direction. Variation in the growth angle θ and includes a stochastic parameter ξ. ξ is a random variable uniformly distributed in preset angle intervals; it is described in the paper as generally being between -10° and 10°. The obvious shortcoming of this model is that it is unable to describe growth in 3D. Additionally, it does not take into consideration the effect of branching that occurs during axonal growth. This model brings value by describing the effect of constrained unidirectional growth of axons, and how that would affect nerve regeneration with the parameter γ.

Here we propose a model to describe nerve regeneration within a nerve guide conduit. Similarly, to the *Razetti et al.* and *Borisyuk et al.* models that came before we will rely on a deterministic and stochastic component to describe axonal growth direction. The model will be mathematically designed on a cylindrical unit system, which best and most easily describes growth in a tube. A COMSOL model is to be developed to reflect the change of the concentration gradient of a particular growth factor (nerve growth factor) in the conduit using this growth factor’s diffusion properties. The COMSOL derived concentration gradient will be transferred to MATLAB. In MATLAB, parameters analogous to β and μ will describe the effect of the concentration gradient on axonal growth. A parameter analogous to γ will reflect the tendency of an axon to sustain a particular growth path when regeneration. A random parameter will characterize the stochasticity of axonal growth trajectories. To this we will add branching probability in the sprouting axon and devise a way to quantify the quality of growth based on “straightness”. We define “straightness” as the percentage of from the proximal end towards the distal end. The smaller the deviation from straightness i.e., the

greater the percentage of growth towards the innervation target, the better. By means of this model, we hope to predict changes in axonal regeneration in a nerve guide conduit in the presence of a growth factor. We hope to show how growth factor supplementation could be used to increase surgical outcomes with NGCs.

Methods

In order to model the progression of growth factor-directed axon growth in simple nerve guide conduits over time, 5 assumptions were used to create a simplified model that accounted for the diffusion of growth factors, the movement of axonal growth cones in response to growth factors, and the branching of axons as during propagation towards the distal end of nerve guide.

The diffusion of growth factors from various initial conditions were modeled using a fluid flow PDE that was simplified with three key assumptions. First, the model assumes that movement of growth factors is determined entirely by molecular diffusion rather than convection since the nerve guide is enclosed and thus net convective flow into or out of the system cannot meaningfully occur. Secondly, the model assumes that axonal growth inside the nerve guide is largely directed by the concentration of a single growth factor added to the conduit, so that the effects of each growth factor can be modelled separately. And third, the model assumes a simple cylindrical nerve guide that therefore contains solid boundaries on all sides at a distance determined by the dimensions of the nerve guide, since nearly all peripheral nerve repair nerve guides in the literature or used clinically use a cylindrical design[8]. From this, discrete approximations of growth factor concentration in the nerve guide as a function of position and time are calculated and sampled for the continuous models of axon growth.

In order to model continuous axon growth, two SDEs were used to model both the change in angular direction of the growth cone and the movement of the growth cone in its current direction at each time step. In addition to the assumptions made in the PDE model of growth factor diffusion, this model makes a fourth assumption that the nerve guide being modeled is a standard hollow cylinder nerve guide with no internal material intended to direct the growth of the axons, since such devices see the majority of real-world use among currently developed nerve guides[8]. The SDE model of growth cone direction uses the gradient in growth factor concentration to reorient the growth cone in the direction up the gradient, with a random stochastic element, at each time step. Similarly, the SDE model of growth cone movement uses the concentration of growth factor around the growth cone in order to determine the distance moved by the growth cone, with a random stochastic element, in the direction the growth cone is currently oriented. The two-dimensional change in position after each time step is then computed from the new orientation and distance travelled at each time step. Since the concentration values used for these SDEs are discrete, the four nearest concentration values generated by the PDE are sampled in order to approximate the continuous concentration at the growth cone's current position.

The last major component of the model of axonal growth is the model of axon branching behavior. Due to the many unknown factors surrounding the mechanisms that drive axon branching, a model that accounts for the chemical signaling that drives branching behavior is not currently possible, and thus this portion of the model makes the fifth and final assumption that axon branching can be effectively modelled by a purely probabilistic model of branching behavior that seeks to emulate the branch patterns observed experimentally[6]. In order to model this branching behavior, a fixed and experimentally determined probability of the axon branching at each time step is used to randomly decide whether not to branch off after each time step in the axon movement model. The growth of this axon is then modelled according to the PDE and SDEs mentioned above until the simulation is completed.

In order to run the SDE model of growth cone movement, a dataset describing the concentration of nerve growth factor at various positions and at each time step must first be generated. To implement the PDE diffusion model and generate this dataset, COMSOL Multiphysics software was used to simulate the transport of a dilute species in a 3D cylindrical environment. The effusion of growth factor into the cylindrical nerve guide conduit was modelled as an exponentially decaying flux of growth factor into the system from the cylindrical walls and/or distal end of the model, depending on the conditions being simulated. The model utilized 3 key parameters taken or calculated from the literature: initial flux values, **B**, the exponential decay time constant, $\boldsymbol{\tau}$, and the effective diffusivity of growth factor, $\mathbf{D_{eff}}$[9,10] . With this COMSOL model, simulations of diffusion were carried out in time steps of 5 minutes for a total simulation time of up to 45 days. The simulation data of the concentration and gradients of growth factor at each model point and time step were then exported, processed to remove non-value entries, and imported into a MATLAB programming environment for use in the SDE growth cone model.

In MATLAB, a stochastic model of the position of the growth cones of a regenerating axon was implemented that used the concentration and gradient data from COMSOL to calculate a change in orientation and a distance travelled at each time step. To begin the model, a random point along the yz-plane, the proximal end of the simulated NGC, and within the nerve guide conduit's radius from the origin is chosen to act as the starting point for the axonal growth cone being simulated. From this point, the SDE models of growth cone movement will determine the angle and position the growth cane after each time step. Since the data set for growth factor contains a discrete set of values, the concentration and gradient at the growth cone position for each time step is approximated by sampling the 8 closest mesh points from the discrete dataset. To accomplish this, an index was assigned to each mesh point in the growth factor data set and searched through to remove all but the 8 points closest to the growth cone on the current time step. The concentration and gradients in each direction were then averaged for all 8 points and used in the subsequent model calculations.

The distance travelled in each time step was calculated according to the equation $NGFrate * C + BaseRate$, as a function of the concentration at the growth cone's position. Both the change in position caused by every unit of concentration, **NGFrate**,

and the rate of growth cone movement without growth factor, **BaseRate**, were parameters taken from the literature[9].

The change in angular orientation at each time step was calculated according to the equation $\theta_{n+1} = (1 - \gamma) * \theta_n + \mu * (\psi_n - \theta_n) + \xi_\theta$, as a function of the gradient in the given angular direction. This equation is composed of three parts that each model a different experimentally observed behavior of growth cones. The first term in the equation, $(1 - \gamma) * \theta_n$, models the tendency of growth cones to reorient themselves in the direction they were initially facing after injury, even in the absence of any known neural growth factors that could direct growth. The degree of this re-orientation is determined by the parameter $\boldsymbol{\gamma}$, which scales this term of the equation and is estimated from experimental data in the literature. The second term in the equation, $\mu * (\psi_n - \theta_n)$, models the tendency of growth cones to reorient themselves up the concentration gradient of neural growth factors, used to calculate $\boldsymbol{\psi}$[7]. Like with the previous term, the degree of orientation change is controlled by a parameter, **μ**, which is estimated from experimental data in the literature[7]. The final term in the equation, ξ_θ, is a stochastic term taken from a normal distribution with a mean of zero and a variance determined by the parameter $\boldsymbol{\alpha}$, which is taken from the literature such that a distribution that matches experimental observations is produced[7]. From the change in angles and the distance travelled at each time step, the new position of the growth cone in cartesian coordinates is calculated and stored for plotting.

In addition to the above calculations, each time step included a fixed probability of the axon branching into two growth cones. The probability of this branching behavior occurring at any time step is set by the parameter $\mathbf{P_{Branch}}$, and then modified based on the total length and time step size of the current simulation. Branches off of the axon are modelled as separate growth cone paths simulated in parallel with each other branch at each time step and with all position data from before the branching time copied from the axon that the cone branched off of. The probability of branching is taken from the literature, though inconsistencies in the literature make this parameter notability more arbitrary than the others in this model[8].

As a part of this model, a cylindrical boundary was defined with dimensions matching the nerve guide conduit being modelled. Any time step in which a growth cone would move beyond the boundary would instead result in the growth cone's new position and orientation being defined as if the cone had travelled along the cylindrical boundary after collision.

Finally, at each time step a measurement would collect of how far the growth cone had travelled overall, and how far it had travelled in the direction of the distal end of the nerve guide conduit. At the end of a simulation, the ratio of the distance travelled toward the distal end and the total distance travelled would be taken and stored as a metric of the "straightness", or tendency to move toward the distal end, of the growth cones.

Model Verification

Axon growth trajectories are largely affected by γ(gamma) and the nerve growth factors in the conduit. Gamma serves as an orientation correction to influence the growth cones to point toward the distal end of the conduit, regardless of the presence of nerve growth factors. Figure 1 below shows the trajectory of axon growth cones without the effect of either gamma or nerve growth factors. The trajectory of the axon is random, without a direct path toward the distal end or straightness to help it grow from one end to the other. The straightness of this axon without any factors influencing its trajectory is .0833 and the fractional length it traveled through the conduit is .0849.

Having only gamma influence the trajectory of the growth cone without any growth factors, Figure 2 shows an obvious increase in straightness and length traveled through the conduit. Gamma helps orient the growth cone to the distal end creating a straightness of .8877 and a fractional length traveled across the conduit of .9060.

Figure 3 shows the effect of having both gamma and nerve growth factors guiding the trajectory of the growth cones, resulting in a larger amount of straightness and length traveled, .9065 and .9419, respectively. As expected, nerve growth factors prove to have an obvious effect on the trajectory of axon growth within a conduit.

Figures 1 and 2 were made using only the matlab model without taking concentration into account. Figure 2 includes gamma as a parameter to show the effect of it on the trajectory of the growth cones. Figure 3 takes the concentration from the comsol model into account. The extracted data from the comsol models include coordinates of the mesh points, concentration at those points, and at different time steps throughout the duration of the experiment. The data is then placed into arrays in the matlab model to plot the axon trajectories at each of those points, resulting in the figures below.

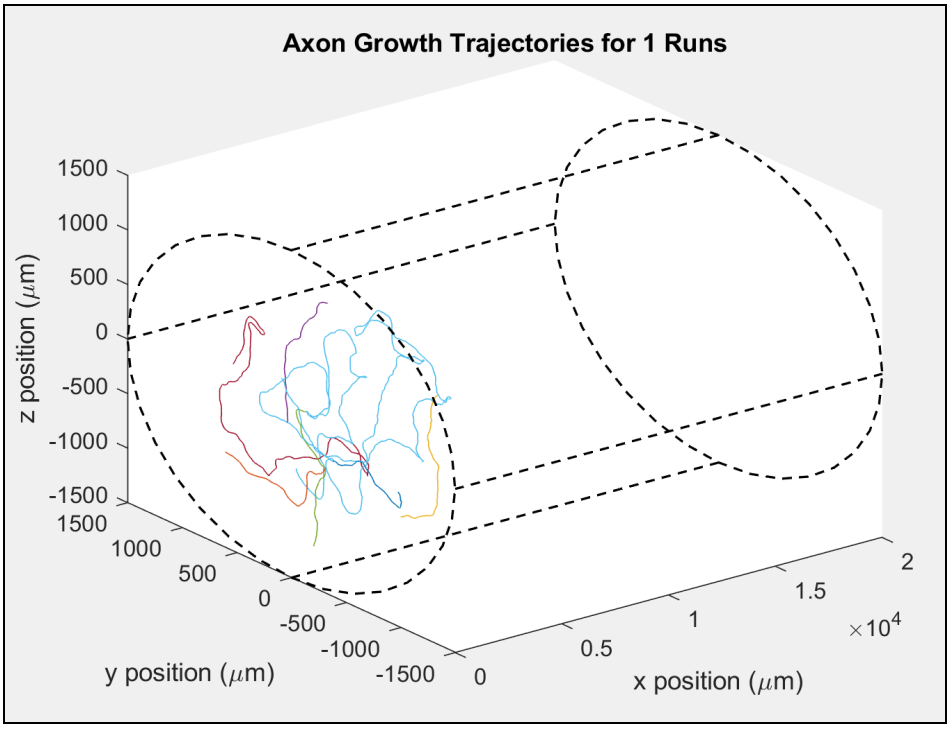


Figure 1. *Axon growth trajectory in a conduit without the effects of the nerve growth factor or gamma. The fractional straightness of this trajectory is .0833 and the fractional length traveled across the conduit is .0849.*

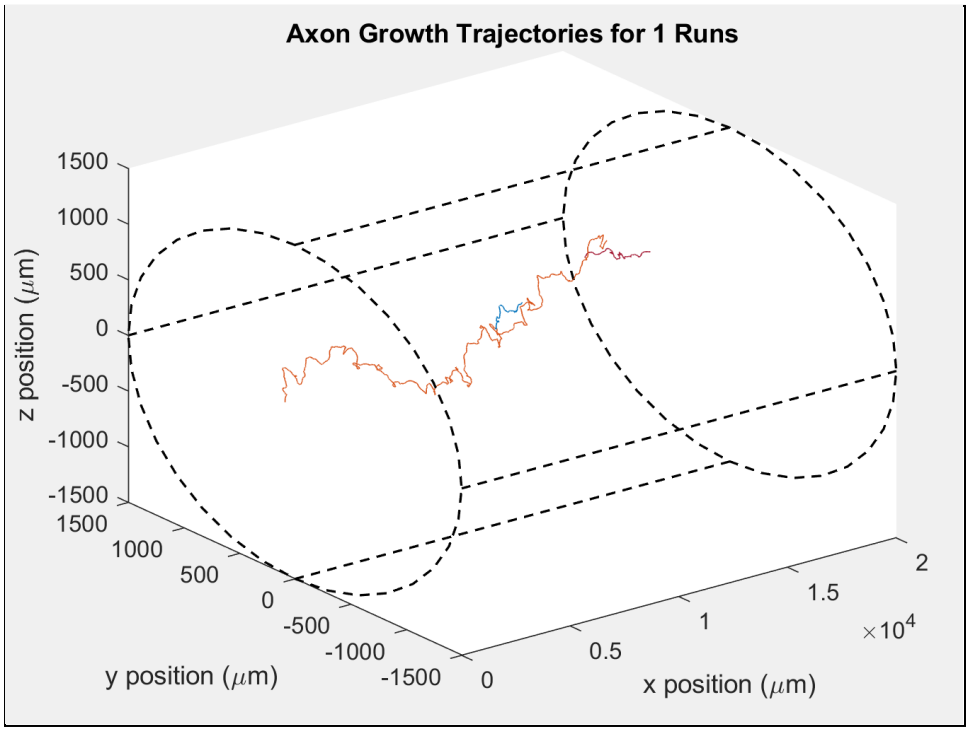


Figure 2. *Axon growth trajectory in a conduit without the effects of the nerve growth factor. Gamma is taken into consideration to drive the trajectory toward the distal end.*

The fractional straightness of this trajectory is .8877 and the fractional length traveled across the conduit is .9060.

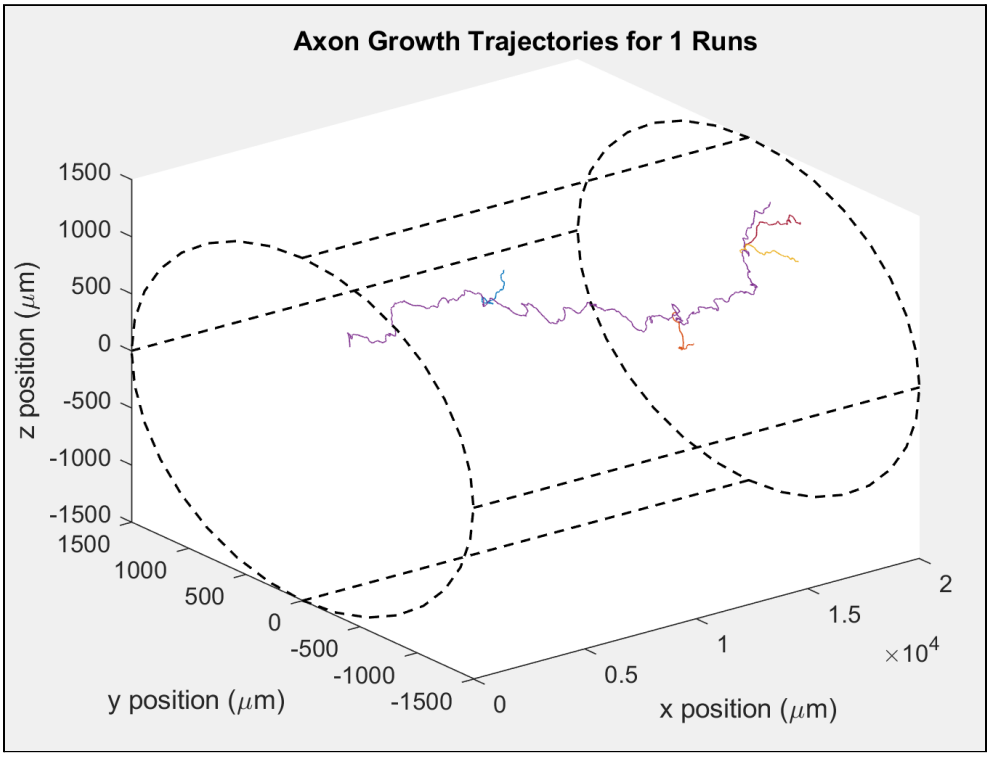


Figure 3. *Axon growth trajectory in a conduit, taking into consideration the concentration of the nerve growth factor and gamma to drive the axon toward the distal end. The fractional straightness of this trajectory is .9065 and the fractional length traveled across the conduit is .9419.*

The COMSOL model for concentration in the conduit uses a decaying exponential equation for the flux.

$$\mathrm{J} = (0.33{*}10^{-15})exp(-\tfrac{1}{2.75{*}3600}t)$$

To get the concentration at the points, the equation is first integrated from 0 to 45 days to get the total concentration.

$$\int_0^{3888000}(0.33*10^{-15})exp(-x/9900)\,dx = 2.75*10^{-12}$$

Then it is multiplied by the surface area of the walls and distal end, and finally divided by volume to get the concentration at the points.

$$(2.75*10^{-12}mol/m^3)(1.96*10^{-4}m^2)/(1.41*10^{-7}m^3) = 3.8*10^{-9}mol/m^3$$

Results

Modeling Nerve Growth Factor Release Schemes:

As no standard flux nor release scheme exists for nerve guide conduits, the NGF flux and the surfaces from which NGF is released were varied to assess the performance of different fluxes and schemes. We defined a release scheme as a set of boundary conditions and initial values in the conduit. For initial conditions, we set the concentration to 0 mol/m^3 at all points. NGF initial flux at a boundary was set to either 0 mol/(m^2·s), 3.35e-16 mol/(m^2·s) (referred to as "Lower Flux") and 3.35e-14 mol/(m^2·s) (referred to as "Higher Flux") and the NGF was released from either the walls along the length of the cylinder, or both the walls along the length of the cylinder and the distal end. Flux would exponentially decay following the function with a time constant of $(2.75*3600)^{-1}$ s^{-1}. This time constant ensured that flux decayed to 0.5% of the initial value after 14 days. The values for initial flux was selected so that once the system reached steady state with the given time constant the concentration in the conduit would be close to either 0.5 ng/ml^3 or 50 ng/ml^3 which were the concentrations of NGF used to measure changes in growth rate in *Turney SG et al*[9]. Figure 1 shows the COMSOL diffusion model of the conduit with the Higher flux on the length of the cylinder and the distal end. Note how the gradient is nearly parallel to the x axis goes from greater concentration on the distal end to lower concentration proximal end.

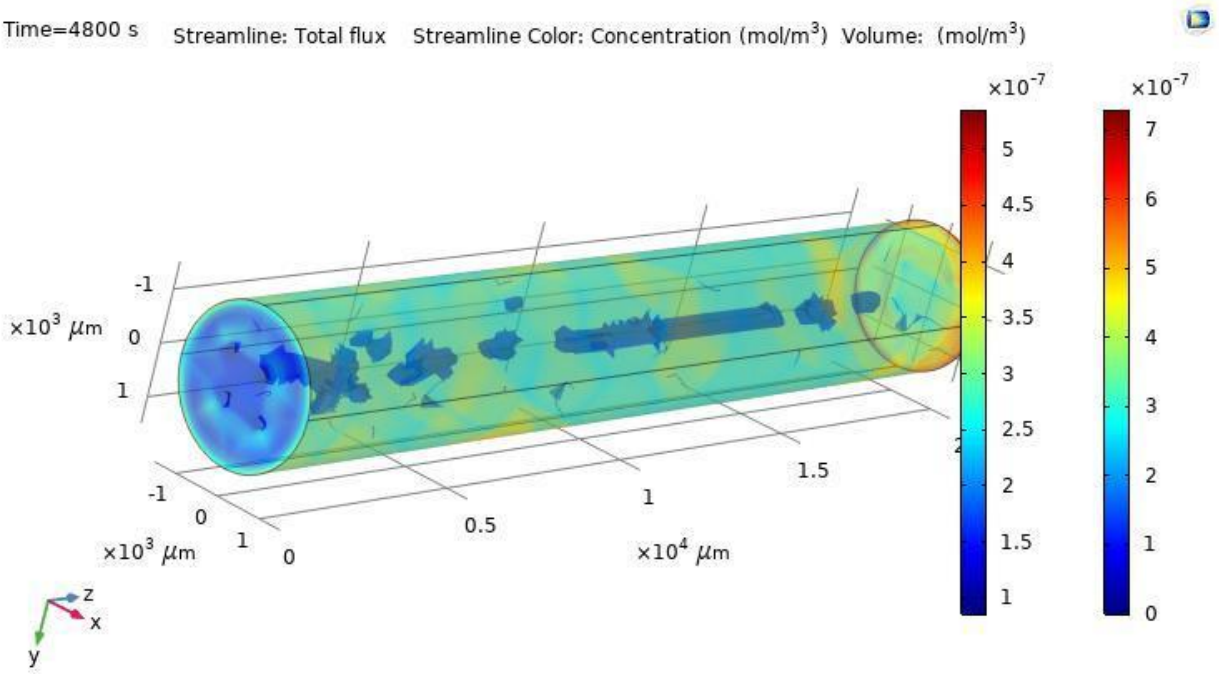


Figure 1. COMSOL Neural Growth Factor Diffusion Model after 4800s. Proximal end is to the left at x=0 and the distal end is to the right at x=2E4. The Higher flux is coming from both the length of the cylinder and from the distal end.

Modeling Axonal Growth Cones Using NGF Concentration and Concentration Gradients:

The concentration and gradient data over time per scheme from COMSOL were moved into arrays in MATLAB and integrated into our axon growth cone model program. At every step the arrays were sampled to find the approximate concentration and concentration gradient at a step's starting coordinate (x_n,y_n,z_n) by finding the mean for these parameters from the 8 closest mesh points. The concentration gradient data would be used to find the azimuthal and polar angles of the concentration gradient gradient's vector. These angles, combined with the mean concentration at the coordinate would inform the change in the growth cone's azimuthal θ_{n+1} and polar Φ_{n+1} angles for the step as well as the absolute magnitude of growth for the step r_{n+1}. We attempted to manufacture ideal gradients and concentrations to maximize growth across the conduit by altering the flux of NGF at the boundaries as well as which boundaries released NGF. This set of modifications was the scheme being tested. Figure 2 shows what the growth trajectories created by our model looked like.

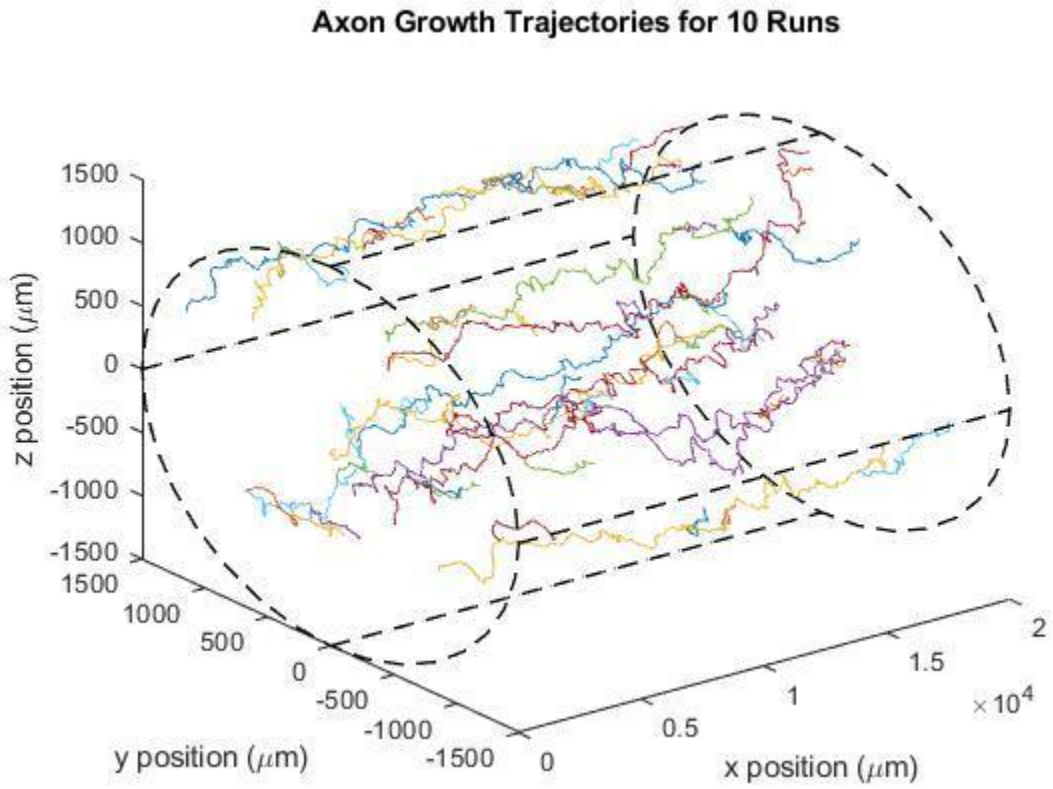
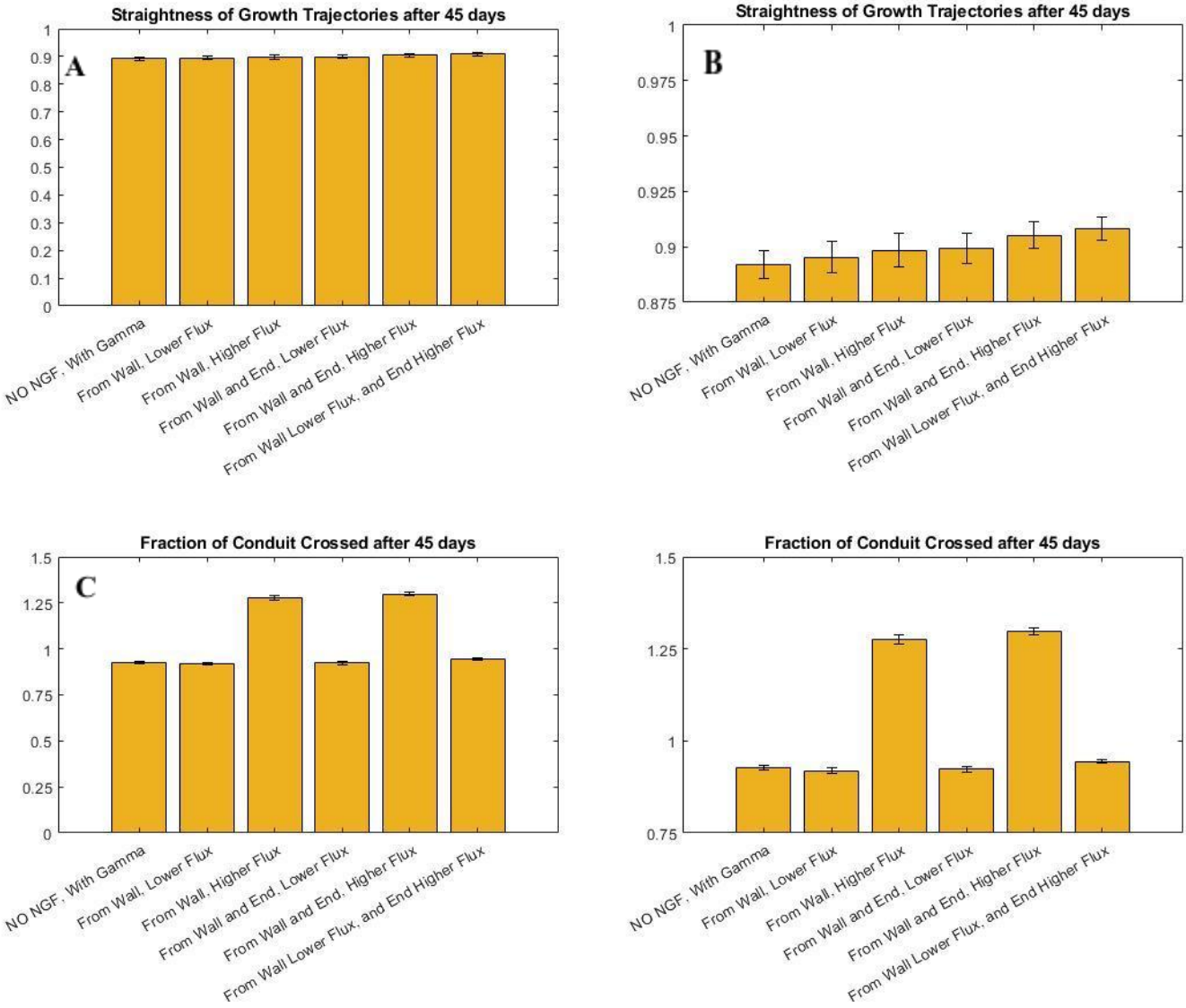


Figure 2. The trajectories for 10 axons modeled using our MATLAB program. The starting point of each axon on the proximal end is randomly generated. The structure represented by the dashed lines is the boundary of the conduit as previously defined. The differently colored appendages to the axons jutting out from the axon are the trajectories of the side branches.

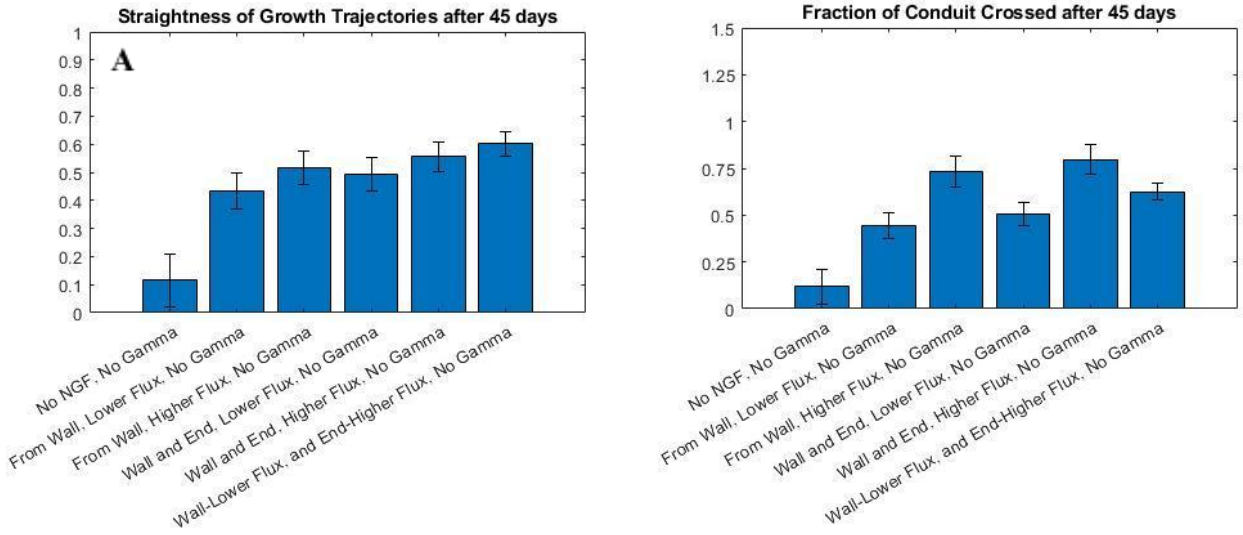
Evaluating Nerve Growth Factor Release Schemes:

The effectiveness of the various fluxes and release schemes were assessed on the mean straightness and fraction of the conduit crossed for 250 trajectories modeled using the fluxes and schemes. The modeled performances of the tested combinations are found on Figures 3a-d.



Figures 3a-d. ***(a)*** *Bar graph of straightness of the 6 schemes tested over 45 days of growth.* ***(b)*** *Close up of the bar graph in 2a to show the minor differences amongst the schemes.* ***(c)*** *Bar graph of the fraction of the conduit crossed by the 6 schemes tested over 45 days. Values above 1.00 indicate that the scheme would have crossed the length of the conduit in under 45 days.* ***(d)*** *Close up of the bar graph in 2c to highlight relative magnitude of the differences amongst the schemes.*

The results from our first set of evaluations demonstrates how influential the gamma parameter is in the straightness of the trajectories, with the concentration gradients having a much more modest influence on straightness. Further it was made clear that the biggest factor in quick traversal of the conduit by the axon was the concentration of NGF available to the axon leading to an increased growth grate. This led us to question how effective the release schemes were on creating a meaningful concentration gradient for the axon to follow. To assess this, we silenced the gamma parameter meaning the only factor influencing change in trajectory was NGF concentration, concentration gradient, and the random noise per step. This produced a notably different result as seen in Figure 4a-b.



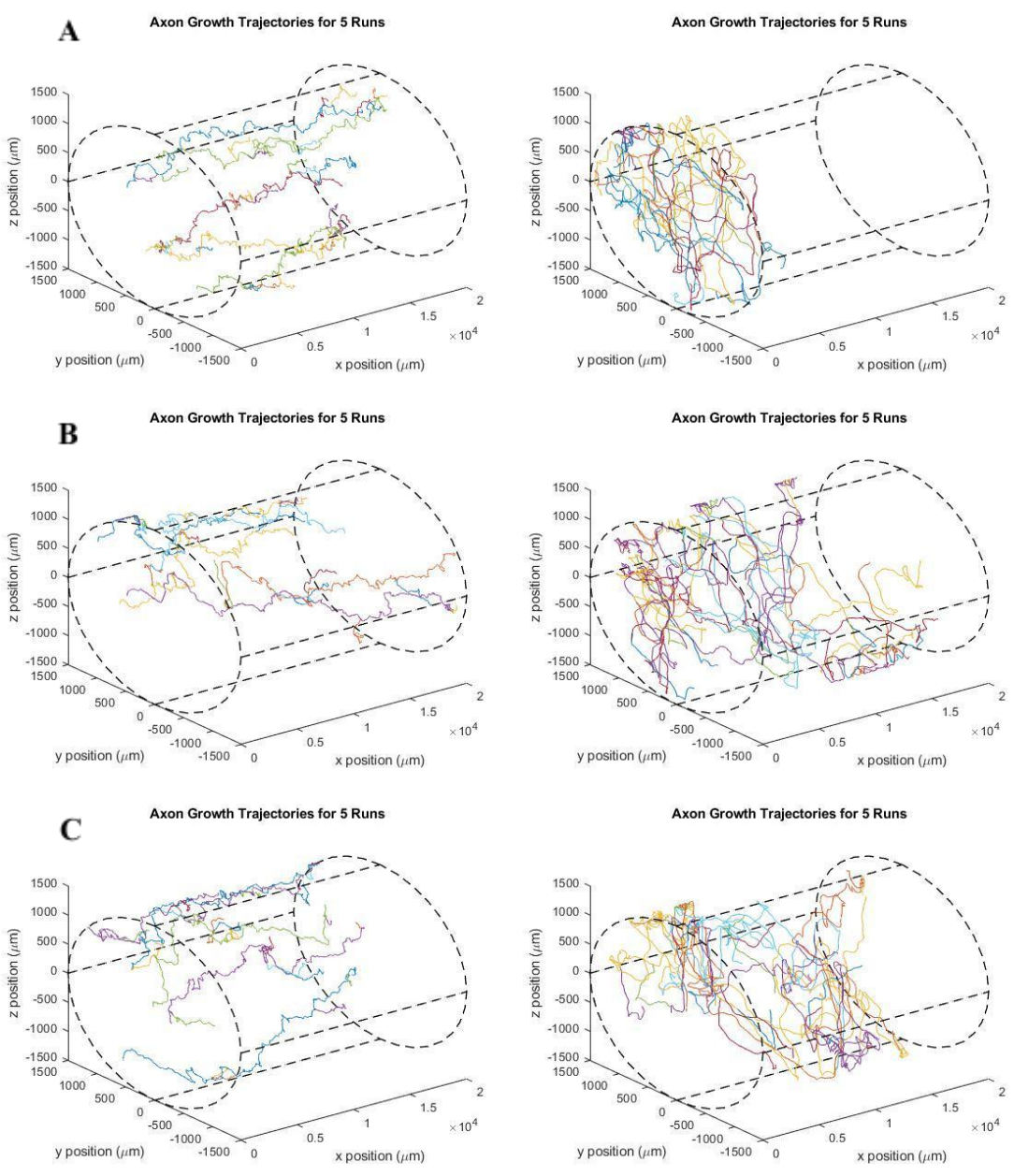
Figures 4a-b. ***(a)*** *Bar graph of straightness of the 6 schemes tested over 45 days of growth with the gamma parameter silenced.* ***(b)*** *Bar graph of the fraction of the conduit crossed by the 6 schemes tested over 45 days with the gamma parameter silenced.*

When gamma is silenced the impact the concentration gradient generated by the scheme has on axonal trajectories becomes much more apparent. Figure 5 shows not only the impact the removal of gamma has on the behavior of individual growth cones, but also how the different release schemes influenced the growth cone trajectories. Table 1 shows the quantitative difference the absence of gamma has.

	Scheme 1	Scheme 2	Scheme 3	Scheme 4	Scheme 5	Scheme 6
Straightness (With γ)	0.8918 (0.0062)	0.8953 (0.0069)	0.8985 (0.0078)	0.8993 (0.0068)	0.9051 (0.0061)	0.9082 (0.0051)
Fraction Crossed (With γ)	0.9269 (0.00065)	0.9188 (0.0071)	1.2753 (0.0113)	0.9228 (0.0070)	1.2975 (0.0088)	0.9439 (0.0053)
Straightness (Without γ)	0.1152 (0.0930)	0.4327 (0.0642)	0.5169 (0.0594)	0.4933 (0.0603)	0.5554 (0.0547)	0.6018 (0.0424)

Fraction Crossed (Without γ)	0.1178 (0.0951)	0.4439 (0.0659)	0.7338 (0.0844)	0.5062 (0.0619)	0.7959 (0.0784)	0.6252 (0.0441)

Table 1. Straightness and Fraction of Conduit crossed for schemes with and without γ. Standard deviation in parenthesis. ***Scheme 1:*** *No Flux.* ***Scheme 2:*** *Lower Flux from length wall.* ***Scheme 3:*** *Higher Flux from length wall.* ***Scheme 4:*** *Lower Flux from both length wall and distal end.* ***Scheme 5:*** *Higher Flux from both length wall and distal end.* ***Scheme 6:*** *Lower Flux from length wall, Higher from distal end.*



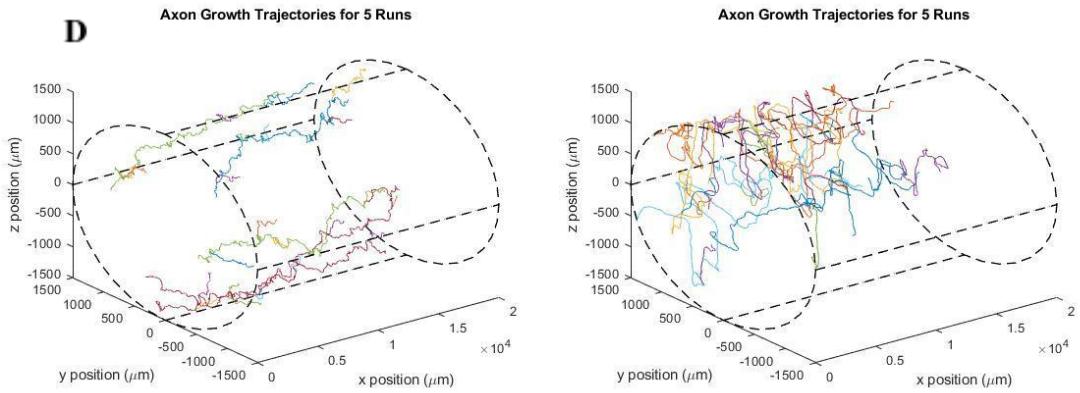
Figures 5a-d. Trajectories with gamma active sit on the left column, while those with gamma silenced sit on the right.

(a) *Comparison of the No NGF scheme with and without gamma.*

(b) *Comparison of the Higher Flux from the length of cylinder scheme with and without gamma.*

(c) *Comparison of the Higher Flux from the length of cylinder and distal end scheme with and without gamma.*

(d) *Comparison of the Lower Flux from the length of cylinder and Higher Flux from the distal end scheme with and without gamma.*



As seen above the absence of gamma causes lowered straightness and a lower fraction of the conduit traversed. This appears to be due in part to the fact that often ψ is not pointing directly to the distal end, but instead is angled partially towards the cylinder wall. This can be seen in Figure 6. Without the effect of gamma, the trajectories' attraction towards the cylinder wall is much greater and thus resulting in a far less straight trajectory. This can be seen by the tendency of the trajectories of schemes lacking γ to persist in the vicinity of the cylinder wall. In turn the main driver of growth towards the distal end is almost entirely the concentration gradient in this instance. This indicates that it is possible that some NGF release scheme can guide an axon towards the distal end of a conduit significantly better than via the effect of gamma alone.

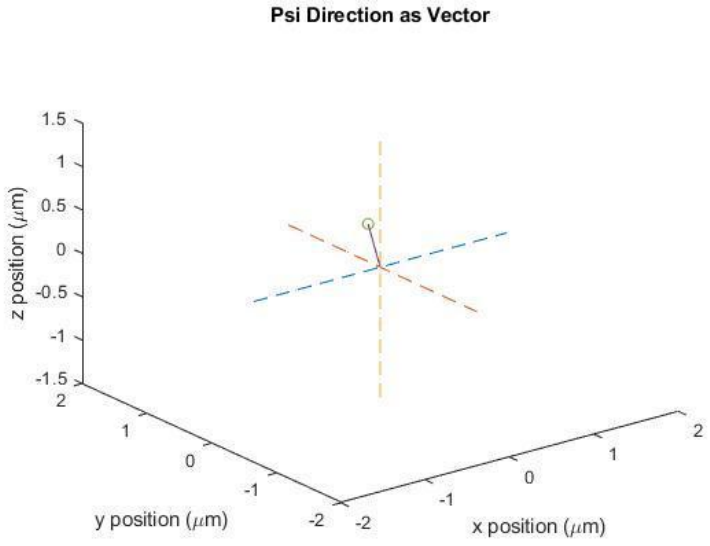


Figure 6. Vector showing gradient direction influencing an individual growth cone trajectory at 210000s. Note that while it does partially point in the positive x direction, it also partially points towards the nearest wall.

Discussion

In order to discuss our level of certainty we need to address how a portion of the parameters were determined from literature. Additionally, we describe here the type of experimentation would increase our LOC and further the significance of the results drawn from our model. First off, a portion of our MATLAB model's equations describing growth cone trajectory (angle at each step during stepwise growth) through the nerve guide conduit rely on parameters that have not yet been experimentally determined in human peripheral nerves. γ is described by *Borisyuk et al.* as the "tendency of an axon to turn towards an angle of 0° - in other words the tendency of the growth cone to orient towards longitudinal growth"[7]. The angle mentioned here is θ, that between the axonal tip and the desired direction of growth (straight line between the proximal and distal end). In our model, θ is between the axonal tip and the x-axis. γ varies between 0 and 1 depending on the type of neuron, and describes the likelihood of an axon to grow longitudinally. The Borisyuk model looks at longitudinally oriented neurons in the spinal cord of a tadpole; it stipulates that ensuing growth should follow the same longitudinal trend thus explaining the need for γ. In our model we use a γ value consistent with the descending interneurons (dIN) of a tadpole. For far more relevance in a clinical setting and accuracy we would need to study the decay of θ towards 0 in the future. This parameter could be determined experiment by studying the deviation of axons from a 0° θ angle when growing. In the absence of any growth factors an axon is constrained to grow unidirectionally over about an arm's length so as to establish a longitudinal growth trend that an axon will try to follow. The constraint is lifted and the axon is allowed to grow unrestrained. The change in orientation θ (angle between the axon and established longitudinal direction) is recorded at equal time intervals for a statistically significant amount of time as in *Katz et al*[11]. The parameter γ can be obtained from fitting the model to match the behavior of θ with time. A stochastic parameter ξ should still be included to address potential "noise" in the change of θ.

Another parameter that needs to be determined experimentally in human peripheral nerves is μ, which (in tandem with ψ, the angle between the growth cone and a chemoattractant) describes the magnitude of the effect of the chemoattractant. Essentially, how much do chemotactic factors influence the trajectory of axonal regeneration. Once again, we use a μ consistent with the descending interneurons (dIN) of a tadpole. For clinical use this model would require a human derived μ; especially since an intended added value to this model is to be able to determine the influence of growth factors on axonal growth, and the ideal concentration for regeneration. To build a data set from which this parameter could be derived we propose an experiment where an axon is constrained to grow longitudinally over about an arm's length so as to establish a longitudinal growth trend that an axon will try to follow as in the previously described experiment. The constraint is lifted and a chemotactic factor source is placed at a known angle and distance from the tip of the axon (ψ). The chemotactic source should have a known concentration that is clinically relevant; a concentration that could be supplemented by a guide conduit or comparable to *in vivo* conditions. The amount of time it takes for ψ to be 0° is measured all the while keeping clear timesteps at which ψ

and the distance of the source from the axon tip is recorded. Similar measurements have been performed in past studies by overlaying a transparent grid on the video image rendered by a phase microscope. The parameter μ can be determined by fitting the change in ψ over time. We do note that such an experiment may yield different μ for different chemotactic factors, and should be tested for a variety of these. Notably those that are being considered for application in peripheral nerve injury treatment. We also expect that results from this experiment will be affected by already established γ (the likelihood of an axon to persistently grow in a certain direction) and ξ_2 (random variations in the angle ψ).

The final parameter that needs to be elucidated for human peripheral nerves is the frequency of occurrence of axonal branching. That is, how often are growth cones bifurcating over a certain distance or a certain amount of time. Our model relies on branching probability, which has not been studied in human peripheral nerves yet. The majority of studies focusing on branching look at the neural tree of the brain. A relatively simple add-on to previously described experiments would enable us to determine this parameter. When determining γ and ψ the branches sprouting from the unrestrained axonal growth portion are counted throughout the duration of the experiment. The frequency of branching can thus be found for axon length or over time. We note that chemotactic factors also likely play a role in branching, and having a control without factors (in the γ experiment set) is key to holistically model branching. Various factors should be also introduced to the growing axon in varying concentrations as well.

We note that axonal growth is heavily influenced by its environment, and that consistency is important. It would be possible for the parameters to change if axonal growth is modeled in a setting for which this model isn't designed. Additionally, a statistically significant number of experiments would need to be done before a parameter sweep could be performed.

The generation of time dependent concentration profiles in the nerve guide, a model of NGF diffusion in COMSOL was used that contained 6 unique internal parameters used to fine tune the model such that it could match growth factor diffusion observed experimentally. Among these parameters, four are largely arbitrary values that must be changed depending on the specific nerve being damaged and the degree of damage. These parameters are L, the length of the nerve guide, W, the width of the nerve guide, T, the total simulation time, and Dt, the time step of the system. Both the length and width of the nerve guide model must be altered to match the dimensions of the nerve guide needed to facilitate nerve regeneration and thus both parameters will scale with the length and width of the damaged nerve. For the purposes of this project, the dimensions of the model nerve guide were set to 20mm in length and 3mm in width, to arbitrarily match a digital nerve's nerve guide. The total simulation time and time step must similarly be fine-tuned to the type and size of nerve guide being modelled since axon regeneration occurs on the order of single digit millimeters per day and thus longer nerve guides must be simulated for longer total spans of time in order for the simulated axons to reach the distal end of the nerve guide. In addition, the time step of the model is limited by the amount of time and computing resources available to run the simulation

as well as the desired model fidelity. Due to the fact that the model needed to be run multiple times in the making of this project, a moderate time step of 5 minutes was used and in order to model growth for a 20mm long nerve guide, an 18 day long total time span was used per simulation. All four of these parameters depend on the specific nerve guide being modelled and are thus arbitrarily assigned based on a digital nerve regeneration model in this project.

The remaining two parameters used in the diffusion model are D, the diffusivity of the NGFs in solution, and C0mol, the peak molar concentration of NGF at the boundary. In the literature, no consistently determined experimental data on the diffusivity of NGFs in humans is available. For the purposes of this project, a diffusivity of 1.26e-10 m^2/sec determined from experiments on mouse models was used. In order to improve the accuracy of this parameter, an experiment could be performed to directly measure effective diffusivity by measuring the concentrations of NGFs at varying depths when added to a solution representative of the nerve guide environment. From this experiment, the diffusivity of NGF can be determined by using an objective function to determine the system parameters from the concentration data set assuming that the model is accurate. Similarly, the molar concentration of NGFs has not been consistently experimentally determined in humans and has only been well documented in models such as mice and rabbits. For the purposes of this project, a peak molar boundary concentration of 3.65e-12 mol/m^3 determined from experiments on mouse models was used. To obtain a more accurate value of this parameter, fluid samples from the proximal and distal ends of implanted nerve guides over time could be taken in order to measure the highest NGF concentration in the standard nerve guide environment. So long as basic nerve guides are used, the peak value of samples from any region in the nerve guide can be averaged out to determine the value of this parameter in humans assuming that the model is accurate.

Taking all of these factors into account we believe to have developed a model that includes all of the components required to successfully describe the trajectory of axons as they regenerate in a NGC in the presence of NGF. We can clearly expect that much more clinical relevance would be obtained from determining parameters that are reflective of axonal regeneration in humans. Until these parameters can be determined from human data our LOC in the accuracy of the model is quite low. This can notably be seen in the strength of the γ parameter as it seems to overpower the effect of the NGF concentration gradient. As a result, the influence of NGF is “downplayed”, which would go against the current clinical research aimed at determining growth factor concentrations that would accelerate and improve axonal regeneration. Additionally, from our validation experiments we see that the axons grow a little over 20mm over the course of 45 days. It is reported in literature that the axonal regeneration in humans hovers around 1mm a day. There is a bit of a discrepancy between the two regeneration rates, which could be due to the base growth rate that we chose or an effect of the parameter values used in our validation experiments. This certainly affects the significance of the conclusions that can be drawn from our model at this point in time.

Having the skeleton of a working model in humans is encouraging, and it certainly holds promise once more accurate parameters have been found. What we do

know for sure from our verification and validation is that the model does respond to the presence of the γparameter and a concentration gradient of growth factors. Without the γ and μ parameters we have a significantly reduced straightness in the axonal trajectory, which is as expected. As expected the location and magnitude of NGF flux affects the velocity of axonal growth. We saw in our validation runs with higher NGF flux that the concentration gradient was settling fast, which increased the velocity of axonal growth by about 0.25. In another run, having NGF elude from the distal end of the conduit also increased the regeneration rate as well as the straightness. The two rather significant conclusions here are: 1.) Higher rate of regeneration and straightness can be obtained from a gradient in growth factor release increased when closer to the distal end of the NGC. 2.) An increased NGF release rate from the NGC walls is also linked to an increased regeneration rate and better straightness. This should give engineers and researchers some direction when they look to supplement growth factors in future NGCs. Although our low LOC in accuracy makes it difficult to interpret the results from the initial NGF concentrations used, it provides some direction as far as the localization of growth factors in an NGC.

As far as future directions this project could take to ever improve fidelity to the biological system it would be great to integrate a scaffold system and add the effect of Schwann cell signaling to the current model. As described in the Background section of this report, a fibrin bridge forms which allows for Schwann cell proliferation and “nesting” in the fibrin cables. To holistically describe the regeneration process it would be key to investigate the effect of the bridge and the Schwann cells. Currently we solely describe axon regeneration in the absence of any scaffolding or other signaling that could direct growth cone trajectories. Ultimately, adding a modulable intraluminal framework would be key to aiding current NGC research as the inclusion various structures made from various materials in the lumen of NGC to improve surgical outcomes are being actively studied in the clinical space[3]**.**

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Acknowledgements

We would like to thank Professor Wendy Thomas for providing us valuable guidance throughout the final project; notably in order to develop a biologically accurate model and how to value the significance of our results.